EMC-21 M - 4 week stored eggs (white) 29 III 73

PROCEDURE FOR FIXATION OF EGG BLASTODERM (revised from procedure of 31 I 73)

Fixation: 8 % glutaraldehyde in 0.1M collidine buffer

Procedure:

1. Cool egg to about 6C in the refigerator before fixation- about 1 hour
2. Break egg into small dish and orient blastoderm up with spoon
3. Cut albumin around blastoderm with fine round pointed sissors and separate from the vitline membrane.
4. Apply a teflon ring over the blastoderm on top of the vitline membrane.
5. Add fixative to the ring and around the outside of the ring and seal with a piece of poly film. Put into refigerator for 1/2-1 hour
6. Cut the vitline membrane around the outside of the teflon ring with fine sissors and lift ring,with blastoderm attached, free from the egg yoke.
7. Remove the excess egg yoke from the blastoderm by holding the ring on edge and wiping off the yoke drop. Under the binocular scope wipe off all the excess yoke from around the blastoderm being careful not to injur the blastoderm cells.
8. Put ring with blastoderm side up back into fresh glut. fixative in refig. and leave 1-2 more hours.
9. Remove fixative and wash with 0.1M buffer for 5min. , change buffer and leave 5 min., change again and leave in refigerator for about 16 hours.
10. Remove the buffer and replace with 1% osmium in 0.1M buffer in refigerator.
11. Leave in osmium for 4-8 hours then rinse in buffer for 1 hour with 2 changes
12. Dehydrate in Acetone series 10,30,50,70,100% and embed in epon.

EMC-21 N - 6 week stored egg (white) - procedure as29 III 73

EMC-21 P - Fresh eggs(white) - " "